Whole Cell Catalysed Kinetic Resolution of 6-Azabicyclo[3.2.0]hept-3-en-7-one: Synthesis of (−)-Cispentacin (FR 109615)

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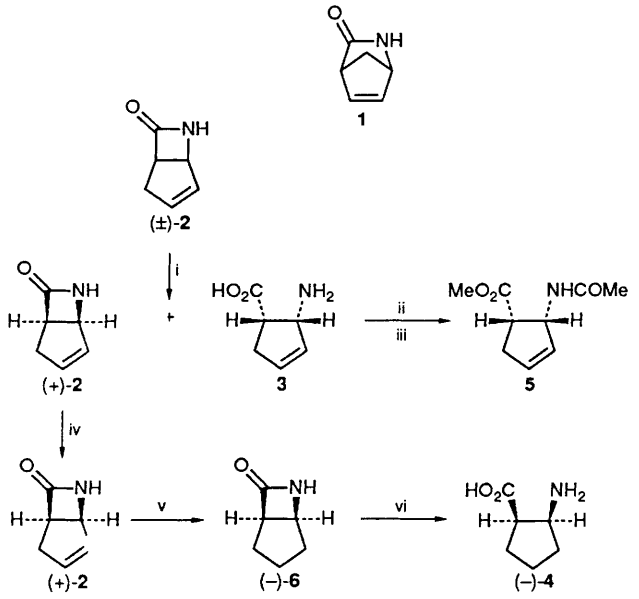
Enantioselective hydrolysis of the β-lactam (±)-**2** using *Rhodococcus equi* provided (1*R*,5*S*)-6-azabicyclo[3.2.0]hept-3-en-7-one (+)-**2**, a precursor of the antifungal agent cispentacin.

Our recent work on the biocatalytic resolution of the carbocyclic nucleoside precursor (±)-2-azabicyclo[2.2.1]hept-5-en-3-one **1** [1] prompted us to investigate a similar strategy for resolution of the isomeric β-lactam (±)-6-azabicyclo[3.2.0]-hept-3-en-7-one **2**.[2] Out interest was heightened by the obvious potential of **2** (or the corresponding amino acid **3**) to provide a precursor of the antifungal antibiotic cispentacin[3] (FR 109615) **4**.[4,5] Herein we report the result of these investigations.

The resolution of (±)-**2** with commercially available isolated enzymes proved unsuccessful. Thus porcine kidney amino-acylase catalysed a non-specific hydrolysis of the lactam while *Aspergillus* sp. aminoacylase, β-lactamases from *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli* or *Staphylococcus aureus*, bovine pancreatic α-chymotrypsin, porcine pancreatic trypsin, proteases from *Bacillus subtilis*, *Bacillus thermoproteolyticus rokko* or *Aspergillus oryzae*, and porcine pancreatic lipase gave little or no hydrolysis.

Resolution was, however, achieved in a highly selective manner using a whole cell preparation ENZA-1 (*Rhodococcus equi* NCIB 40213), an organism which had previously been utilized in the resolution of **1**. Thus, incubation of the cells at 20 °C in water buffered to pH 7 with the lactam until *ca.* 45% hydrolysis occurred gave amino acid **3** which was converted without purification into the corresponding methyl ester acetamide **5** (38% yield based on racemic lactam) (Scheme 1). This material was shown to have an enantiomeric excess (ee) of 96% by gas chromatographic analysis on a Lipodex-D column.

The recovered lactam was then reincubated under the same conditions until the ee was >99%, as assessed by GC analysis, giving a 40% recovery (based on racemic starting material) of



Scheme 1 *Reagents and conditions:* i, ENZA-1, pH 7, 20 °C [(+)-**2**, 53%, 75% ee]; ii, $(MeO)_2CMe_2$, MeOH, HCl; iii, Ac_2O, pyridine, CH_2Cl_2 [38%, from (±)-**2**; 96% ee]; iv, ENZA-1, pH 7, 20 °C [40% from (±)-**2**; >99% ee]; v, H_2, Pd/C, EtOAc (95%); vi, HCl, H_2O (95%)

(+)-**2**. The lactam (+)-**2** was hydrogenated to give the fully saturated analogue (−)-**6** which was, in turn, hydrolysed to give the corresponding amino acid (−)-**4**. This material displayed a similar optical rotation [α] −8 (*c* 1, H_2O)† to that reported for natural cispentacin, $[\alpha]_D$ −10.7 [3] and −8.9 [4,5] (*c* 1, H_2O), which is reported to have 1*R*,2*S* stereochemistry.[5] On this basis we can assign 1*R*,2*S*-stereochemistry to the lactams (+)-**2** and (−)-**6**.

It is interesting to note that ENZA-1 shows poor hydrolytic activity towards (±)-**6**.

Experimental

Enantioselective Hydrolysis of the Lactam **2**.—*Rhodococcus equi* NCIB 40213 (700 mg of paste) was suspended in phosphate buffer (0.05 mol dm^{-3}; pH 7) and 6-azabicyclo[3.2.0]hept-3-en-7-one **2** (340 mg, 3.12 mmol) was added. Stirring was continued at room temperature for 142 h after which the cells were removed by centrifugation. The supernatant was extracted with dichloromethane (4 × 100 ml) and the combined organic layers were dried ($MgSO_4$) and concentrated. The recovered lactam (197 mg) was reincubated with *Rhodococcus equi* (280 mg) in buffer (36 ml) for a further 170 h and then recovered as above. Column chromatography over silica using ethyl acetate as eluent gave (+)-6-azabicyclo[3.2.0]hept-3-en-7-one (+)-**2** (137 mg, 40%) as a white solid; m.p. 76–77 °C, $[\alpha]_D^{20}$ (*c* 0.4, $CHCl_3$) 37; ν_{max}/cm^{-1} ($CHCl_3$) 3414 and 1754; $\delta_H(CDCl_3)$ 6.25 (1 H, br s, NH), 6.12–5.85 (2 H, m, 3-H and 4-H), 4.59–4.42 (1 H, m, 5-H), 3.85 (1 H, ddd, *J* 9.8, 3.5, 3.5, 1-H) and 2.90–2.25 (2 H, m, 2-H_2).

† Units for $[\alpha]_D$ expressed in 10^{-1} deg cm^2 g^{-1}.

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